BIOSCHAMP's lessons learnt

BIOSCHAMP aimed to develop casing soils for mushroom production (alternatives to peat, which is the traditional casing soil) and embed them with a microbial biostimulant (to substitute chemical pesticides). Both innovations were to be analyzed in laboratory, semi-commercial, and commercial trials. Only the best developments reached commercial trials. These were performed by four growers, covering three production styles:

- ✓ **Southern Europe:** Growers use phase II substrate from plastic film bags in their growing units. Approximately 900 bags can be placed in each growing unit, each bag weighing 20 kg. This type of mushroom cultivation on three levels is filled and covered by hand. Climate control is semi-automatic.
- ✓ **Northern Europe:** Growers use phase III compost, prepared in bulk at composting facilities. Filling of said compost and covering with casing soil is done mechanically at mushroom production sites. Facilities are equipped with an automatic system for climatization and aluminium shelves at five different heights.
- ✓ **Organic production:** Compost is based on pure horse manure (bags of 18 kg) and applied using traditional composting methods. There are three levels of shelves with a total capacity of 900 bags. Both rooms filling and casing soil application are done manually. Climate control is semi-automatic.

A summary of lessons learned throughout the 4 years of the project is presented below.

On alternative casing soils

BIOSCHAMP started studying five materials: grass fibers sourced from a natural area, grass fibers sourced from an urban area, sphagnum moss, spent casing (i.e., used peat), and green compost. Both types of grass fibers were subject to NewFoss proprietary process before testing. Thus, we will refer to them as NewFoss natural grass and NewFoss urban grass.
Tests were performed using a combination of each material with peat in a 1:1 ratio, meaning 50% of volume was peat, and the remaining 50% was the corresponding alternative material. We will refer to these mixtures by the name of the alternative material, unless stated otherwise. Based on our promising results, we also performed preliminary trials with higher percentages of peat replacement. Our studies used as control 100% peat (i.e., traditional casing soil).

Main takeaways: NewFoss urban grass fibers and sphagnum moss combined with peat in a 1:1 volume ratio achieved promising levels of productivity (kg mushroom/m^2) compared to traditional casing, and mushroom quality was virtually identical. Through these promising results, BIOSCHAMP showed it is feasible to substitute half of the peat used as mushroom casing for non-fossil resources while achieving comparable mushroom production. These advancements represent a significant step towards a more sustainable mushroom industry. For these solutions to reach the market, further research is needed e.g., on how to tailor these alternative casing materials to achieve maximal productivity in each production style, and on the economic viability and impact on mushroom growers' profits.

Specific findings are presented herewith:

- Laboratory scale trials compared traditional casing to five alternative materials. In these trials, it was possible to replace up to 50% of peat without decreasing lab-scale productivity. The only exception was spent casing. However, a higher level of replacement bore an increased risk for diseases and loss of productivity. Most of the alternative casing materials required steaming as a sanitizing step, which would increase the cost of the potential product.
- In semi-commercial trials, NewFoss urban grass and sphagnum moss achieved mushroom productivity comparable to traditional casing. Thus, it is possible to replace 50% of peat in traditional casing by NewFoss urban grass or by sphagnum moss at semi-commercial scales. At this scale, crop management when using NewFoss urban grass or sphagnum moss was comparable to that of traditional casing. This is expected to facilitate adoption of both alternative casing materials by growers. Factsheets on how to use NewFoss urban grass and sphagnum moss can be found in D5.2.
- Prior to commercial trials, BIOSCHAMP tested steamed and non-steamed NewFoss urban grass and sphagnum moss. Disease incidence was comparable to traditional casing in all cases. Consequently, commercial trials were performed with non-steamed casings to avoid the economic and environmental burden of steaming (e.g., due to high energy requirements).
- Commercial trials showed promising outcomes on productivity, precocity and quality of mushrooms grown with NewFoss urban grass and with sphagnum moss. Results varied based on production style.
- Commercial trials showed that NewFoss urban grass and sphagnum moss require a different watering treatment than traditional casing: more frequent watering using less volume per watering is recommended to reduce bacterial blotch incidence.
- Levels of 1-octen-3-ol and 3-octanone (volatiles hindering productivity and precocity) were comparable among traditional casing, NewFoss urban grass and sphagnum moss used in BIOSCHAMP's commercial trials.
- Given the promising results of BIOSCHAMP's commercial trials with NewFoss urban grass and sphagnum moss replacing 50% of peat, additional trials with higher peat replacement were performed. Results substituting up to 75% of peat were promising. Further studies and replicates are required to confirm this behavior, given it differs from our experience in laboratory trials (see above). Possible causes of this discrepancy are differences in environmental conditions.
- Additionally, small-scale trials testing performance of NewFoss urban grass and sphagnum moss on brown mushrooms were performed in one trial location as a prospective experiment. Results showed that the productivity of brown mushroom species was 11% higher than that of white mushrooms, with 50% NewFoss urban grass + 50% peat being the best performing casing.
- A study of the physicochemical parameters of NewFoss urban grass and sphagnum moss mixed with 50% peat showed that they have (1) a suitable water-holding capacity for mushroom production, (2) protect the compost layer against dehydration, (3) provide an optimal environment for the growth of mycelium, (4) provide a constant acidity, (5) have a good structure and suitable adhesive strength, and (6) are free of diseases and safe to use.
- NewFoss urban grass and sphagnum moss casings (without biostimulant) fulfil the EU Regulation 2009/1009 in terms of (1) composition (absence of listed pathogens) and (2) presence and limits of heavy metals (a multiresidue analysis showed detected compounds comply with limits in mushrooms).

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On biostimulants

BIOSCHAMP studied various strains of *Bacillus velezensis* and *Pseudomonas* spp. for their biostimulant potential, i.e., their ability to increase crop productivity and reduce disease incidence. Our goal was to test said strains at laboratory, semi-commercial, and commercial trials. However, despite promising results in laboratory trials, they did not show biostimulant activity at semi-commercial trials, thus commercial trials were not attempted.

Main takeaways: The biostimulant activity of *B. velezensis* is likely to be caused by the production of fengycins and other antimicrobial compounds. This species inhibits mushroom parasites without negatively impacting mushrooms, thus postulating as a promising biostimulant for mushroom production. However, we did not observe biostimulant activity when bacteria were produced at large scale (100 L). Further research is required to achieve industrial-scale production of this microorganism while retaining biostimulant activity. Moreover, although *B. velezensis* could be isolated from mushroom casing, it did not maintain a high population density when introduced into mushroom growing conditions.

Specific findings are presented herewith:

- → *Pseudomonas* spp. did not present positive results regarding biostimulant potential in semi-commercial trials. Thus, research focused on *B. velezensis*.
- → LC-MS/MS analysis showed the strains of *B. velezensis* produced fengycins – lipopeptides that disrupt cell membranes of specific fungi. As *B. velezensis* was found to inhibit mushroom parasites without having deleterious effects on mushrooms, fengycins are promising natural biocides for mushroom cultivation.
- → BIOSCHAMP developed TaqMan probes and a bioreporter strain to screen for *B. velenzensis* in casings. Results from laboratory trials showed these strains have a low persistence in crop – after two weeks, viable cells were absent. This lack of persistence is consistent with other agricultural applications of *B. velezensis*.
- → Inconsistent effects of *B. velezensis* on yield in laboratory trials indicated that dose, timing, and viability affect the performance of this microorganism.
- → Semi-commercial trials tested *B. velezensis* with NewFoss urban grass and sphagnum moss. When compared to said alternative casings without biostimulant, the addition of *B. velezensis* did not have a significant effect on the productivity, precocity or microbiome of the casings.
- → Based on phospholipid fatty acid (PLFA) analysis, conditions during the cultivation period seem unfavorable for Gram-positive bacteria, such as *B. velezensis*, relative to Gram-negative bacteria such as *P. fluorescens*.
- → *B. velezensis* produced in 5 L bioreactors presented biostimulant activity. When production was scaled up to 100 L bioreactors, cells were not viable and biostimulant activity disappeared. A variety of growing conditions and antifoams were tested without significant effect on viability or biostimulant activity.
- → As an alternative to *B. velezensis,* we studied the biostimulant activity of a cocktail of amino acids and of *Azotobacter* strains in small-scale trials. Disease incidence and productivity were comparable to control (untreated).

On sustainability of the mushroom sector

BIOSCHAMP analyzed the presence of pesticides in 136 straw samples from 6 countries. We expected to find pesticides in samples of conventional straw. However, we also found pesticides in samples of organic straw.

- The group of active substances most detected was fungicides, showing that measures taken to reduce the presence of pesticides in cultivated mushrooms are not 100% effective.
- Composting straw reduces or even eliminates the presence of these active substances.
- Pesticides can reach mushrooms via straw or products used in mushroom cultivation, sometimes exceeding the maximum residue limits (MRLs).

We also performed a social life cycle assessment (sLCA) and an environmental LCA on using NewFoss urban grass and sphagnum moss versus traditional casing. The LCA focused on GWP100, terrestrial acidification, marine and freshwater eutrophication, and use of fossil resources and water.

- Our sLCA showed that using NewFoss urban grass could bring about small shifts in employment along the chain - away from the peat harvesting region. These changes do not necessarily lead to more local employment at the mushroom production stage, given commercial production is partly based on migrant workers. Moreover, there are social risks associated with transport and use of energy (e.g., diesel oil in Spain). Overall, using both BIOSCHAMP alternative casing materials is linked to higher costs for mushroom and casing producers.
- Our LCA showed using NewFoss urban grass reduces the use of fossil resources (peat) but slightly increases the remaining impacts. Oppositely, using sphagnum moss leads to a more substantial decrease in fossil resource use and a slight decrease in other impacts.

Next steps

Research and policy gaps to be tackled

Main takeaways: For the mushroom industry to adopt BIOSCHAMP solutions, efforts should be dedicated to expand current EU regulation and fill in existing knowledge gaps. Key policies to be modified are EU Regulation 2018/848 (which currently prevents adoption of BIOSCHAMP results by organic mushroom producers) and EU Regulation 2019/1009 (which forbids commercial use of bacterial strains studied in BIOSCHAMP as biostimulants). Further lines of research include economic assessment and design of the operational chain of both BIOSCHAMP alternative casing soils, scalability of biostimulant production, monitoring techniques for *B. velezensis*, production of fengycins, and transferability of active substances present in straw. Stakeholders should be informed about the latter.

Specific findings are presented herewith:

- EU Regulation 2018/848 rules acceptable casing materials for organic production. NewFoss urban grass and sphagnum moss are not included, thus organic growers cannot use them nowadays. Adding these two materials to the approved list of casings would allow all mushroom growers to adopt them, reducing the demand for peat. BIOSCHAMP will present this request to the expert group for technical advice on organic production (EGTOP).
- Microorganisms studied in BIOSCHAMP (*Bacillus* spp., *Pseudomonas* spp.) are not currently included in EU regulation 2019/1009 as biostimulants: it lists only *Azotobacter* spp.,

mycorrhizal fungi*,* *Rhizobium* spp. and *Azospirillum* spp. If a viable and effective biostimulant is obtained, its registration and application would take time, since it would require changes in legislation.

- Operational aspects of the commercialisation of alternative casing soils (stability, best shipment method, etc.) must be further analysed.
- Economic assessments of NewFoss urban grass and sphagnum moss are required to fully understand the feasibility of large-scale industrial adoption, especially under the light of our sLCA findings. This research should include key aspects such as:
  - Costs of production, logistics, use and disposing.
  - Effect on mushroom growers' profits, clearly segmenting production dedicated to fresh and to canned mushrooms.
- Considering the good results of brown mushroom production, NewFoss urban grass and sphagnum moss could be studied as casing materials for other kinds of mushrooms.
- Developing stable, scalable biostimulants for mushrooms requires more time and research. This includes growing conditions and antifoams for *B. velezensis* production at 100 L and larger bioreactors. Downstream methods also need further study, especially drying methods to obtain viable cells.
- Fengycins may be promising mycroparasite-specific fungicides. Further research could be performed to assess the feasibility of producing fengycins by fermentation, and into the antifungal activity of specific fengycins.
- It is necessary to develop alternative methodologies to measure the presence of *B. velezensis*. Since it is a sporulating bacterium, DNA measurements may be inaccurate due to low efficiency DNA extraction from spores.
- Awareness should be raised among the mushroom sector about the presence of active substances in straw and the need to monitor them in the materials and in mushrooms. This is especially relevant for the organic mushroom sector.
- Research should be conducted to determine if active substances present in straw are transferred to the final product.
- This project entailed shipping of various kinds of samples to up to countries in and out of the European Union. A defined, common protocol of sample treatment pre- and post-shipment, as well as a common shipment method are expected to decrease the differences among samples.